REMARKS

The Official Action dated May 18, 2001 has been carefully considered. It is believed that the amended claims submitted herewith and the following comments represent a complete response to the Examiner's rejections and place the present application in condition for allowance. Reconsideration is respectfully requested.

Drawings

We will submit the necessary formal drawings once we receive an indication that the application is allowable.

Specification/Informalities

As requested by the Examiner, the application has been amended in order to include reference to the prior application.

The Examiner has requested that the heading "Detailed Figure Legends" on page 19 be replaced with "Brief Description of the Drawings". However, we point out that the Brief Description of the Drawings is given on page 4 and that the description on page 19 is a detailed figure legend providing more information on the figures. As a result, no amendment is necessary.

We do not know why the United States Patent Office does not have a copy of the abstract of the disclosure as it is present on the cover page of the published application that was submitted to the United States Patent Office by the International Bureau. However, in order to comply with 37 CRF 1.72(b), a new Abstract is being submitted herewith.

35 U.S.C. §112, second paragraph

The Examiner has objected to claims 1-19 under 35 U.S.C. §112, second paragraph as being indefinite.

In particular, the Examiner is of the opinion that the term "introducing into a host cell an expression vector" found in claim 1 is unclear. In response, this expression has been replaced with "transforming a host cell with an expression vector" as requested by the Examiner.

The Examiner is of the opinion that the expression "the polypeptide" in claim 5 lacks antecedent basis. In response, this expression has been replaced with "the recombinant polypeptide" as requested by the Examiner.

The Examiner is of the opinion that claim 27 is confusing as "SEQ.ID.NO.2" is an amino acid sequence. In response, SEQ.ID.NO.2 has been replaced with "SEQ.ID.NO.3" which is a nucleic acid sequence.

In view of the foregoing, we respectfully request that the objections to the claims under 35 U.S.C. §112, second paragraph be withdrawn.

35 U.S.C. §112, first paragraph

The Examiner has objected to claims 1-3, 5-22, 23-30 and 41-44 under 35 U.S.C. §112, first paragraph as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skill in the relevant art that the inventors have possession of the invention at the time the application was filed. We respectfully disagree with the Examiner for the reasons that follow.

As the Examiner has noted, claims 1, 2, 3, 20, 21, 22, 41 and 42 are directed to a genus of nucleic acids encoding autocatalytically maturing zymogens comprising a pro-peptide from an autocatalytically maturing zymogen fused to a heterologous polypeptide. The Examiner acknowledges that the specification teaches seven representative species of such pro-peptide but is of the opinion that seven is not sufficient to support the full scope of the claims. We respectfully disagree with the Examiner and submit that his position is contrary to the guidance given in the Revised

Interim Guidelines for Examination of Patent Applications under 35 U.S.C. §112, paragraph 1, "Written Description Requirements". In particular, the guidelines clearly state that the "written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice ... or by disclosure of relevant identifying characteristics ..." We submit that the present application meets both of these criteria.

We submit that the disclosure of seven species within the genus of nucleic acids encoding autocatalytically maturing zymogens is clearly sufficient to demonstrate to one of skill in the art that the Applicant was in possession of the necessary common attributes possessed by the members of the genus. It is noted that the written description guidelines do not provide a numerical value for what constitutes a "representative number of species" although it is noted that in practice, two or three species within the genus have generally been accepted by the United States Patent Office as being representative of a genus.

In addition to disclosing seven representative species within the genus, Applicant also provides a sufficient disclosure of relevant identifying characteristics so that one of skill in the art would know that Applicant had possession of the claimed invention. In particular, autocatalytically maturing zymogens are well known in the art as described on page 3, lines 1-16 of the application. The identifying characteristic of an autocatalytically maturing zymogen is its ability to be processed to its active form without requiring an additional specific protease and that the mature form of the zymogen can assist in a cleavage reaction. (See page 5, lines 26-28 of the application.)

In view of the foregoing, we respectfully request that the claims meet the written description requirements and we request that the objection under 35 U.S.C. §112, first paragraph, Written Description be withdrawn.

The Examiner has also objected to claims 1-30 and 41-44 under 35 U.S.C. §112, first paragraph alleging that the specification does not reasonably provide enablement for a method of preparation of a recombinant polypeptide by transforming a host cell with a nucleic acid encoding any pro-peptide derived from an autocatalytically maturing zymogen upstream of a nucleic acid encoding said recombinant polypeptide, expressing any pro-peptide fusion protein and altering the environment in order to cleave the pro-peptide from the recombinant polypeptide or a chimeric nucleic acid sequence therefor and compositions thereof. We respectfully disagree with the Examiner for the reasons that follow.

On page 7 of the office action, the Examiner alleges that the specification does not enable five features which are identified as (A)-(E). We will address each of these in turn below.

(A) The Examiner is of the opinion that the *in vitro* or *in vivo* environmental, pH, salt and/or temperature conditions that will result in specific cleavage of any pro-peptide derived from an autocatalytically maturing zymogen from the recombinant polypeptide without affecting the recombinant polypeptide activity are not established. We respectfully disagree and submit that one of skill in the art having chosen a particular pro-peptide/heterologous protein combination would be readily able to determine the optimal conditions for the cleavage reactions. Such determination of appropriate reaction conditions permitting proteolysis, is part of a routine procedure a person of ordinary skill in the art wishing to practice the subject invention and having read the disclosure expects to carry out, and will be able to perform without undue experimentation. Further, various suppliers for proteolytic cleavage systems provide information that would assist one of skill in the art in preparing the optimal conditions.

(B) The Examiner is of the opinion that the ability to specifically cleave any pro-peptide from an autocatalytically maturing zymogen without nonspecific cleavage of the recombinant polypeptide is not established. We point out that the claims do not preclude some non-specific cleavage of the heterologous protein. However, we

submit that once one of skill of the art has optimize the cleavage reaction as discussed above under (A), such non-specific cleavage is generally very limited. Further, Applicant has tested many proteins and has not observed substantial non-specific cleavage of any of the proteins.

(C) The Examiner is of the opinion that the predictability of pro-peptides that can be cleaved by zymogens that are heterologous to the pro-peptide and/or that can provide further autocatalytic cleavage in the absence of the mature portion of the zymogen from which the pro-peptides are derived is not well-established. Applicant has shown that pepsin and Aspergillus saitoi acid protease (Sigma 2143) are able to assist in a cleavage reaction using the pro-chymosin sequence linked to hirudin.

(D) The Examiner is of the opinion that the general tolerance of a fusion protein comprising a pro-peptide derived from an autocatalytically maturing zymogen to modification and extent of such tolerance is not well established. We submit that it would be clear to a person of ordinary skill in the art that the tolerance for any such mutations would be evaluated as per the routine procedures described under (A) above and as described in the specification, for example, on page 11, lines 30-34.

(E) The Examiner is of the opinion that the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful. As suggested previously under points (A)-(D), one of skill in the art could readily select a pro-peptide/heterologous peptide combination and develop the necessary conditions in order for the invention to work, such development requiring no undue experimentation.

In summary, we submit that the application does provide sufficient enablement for the claims and it would be unfair to the Applicant to limit the claims to the conditions used in the specific embodiments. One of skill in the art could readily determine the proper conditions for carrying out the method using other pro-peptide/heterologous peptide pairs.

In view of the foregoing, we respectfully request that the objections to claims 1-30 and 41-44 be withdrawn.

35 U.S.C. §102

The Examiner has also objected to claims 1-7, 9-13, 15, 19-26, 28-30 and 41-44 under 35 U.S.C. §102 as being anticipated by Moloney (WO 96/21029). We respectfully disagree with the Examiner for the reasons that follow.

The claims of the present invention relate to a method for the preparation of a recombinant polypeptide wherein the recombinant polypeptide is prepared as a fusion protein with a pro-peptide derived from an autocatalytically maturing zymogen which allows the pro-peptide to self-cleave and release the recombinant polypeptide. The claims specify that the nucleic acid sequence encoding the recombinant polypeptide is located immediately downstream of the nucleic acid sequence encoding the pro-peptide. In contrast, Moloney is concerned with a process for the production of recombinant polypeptides on oil bodies. The method involves preparing a fusion protein comprising the recombinant protein and an oil body protein to facilitate targeting of the fusion protein to the oil body.

Moloney does address separation of the recombinant protein from the oil body protein. However, this involves the inclusion of a conventional enzymatic recognition site (for example, a factor Xa cleavage site) or a chemical cleavage site (for example a cyanogen bromide cleavage site) between the oil body protein and the recombinant protein of the fusion protein. Moloney does not anticipate that a pro-sequence of an autocatalytically maturing zymogen may be employed to facilitate cleavage. In a specific embodiment in Moloney, the heterologous protein is an enzyme such as chymosin. However, we respectfully submit that a chimeric nucleic acid sequence consisting of chymosin linked to an oil body protein does not fall within the scope of the claims of the present application which specify that the heterologous polypeptide must be immediately downstream of the pro-peptide. If the Examiner is comparing

the Moloney chymosin example with the present claims, then the oil body protein must be the "heterologous polypeptide". However, in Moloney, the oil body protein can only be (1) upstream of the pro-peptide sequence in the chymosin, or (2) downstream of the pro-peptide but with the mature form of the chymosin intervening. In no case is the oil body protein immediately downstream of the pro-peptide sequence. Therefore, Moloney does not anticipate the present claims.

In view of the foregoing, we respectfully request that the objections to the claims under 35 U.S.C. §102(a) as being anticipated by Moloney be withdrawn.

35 U.S.C. §103

The Examiner has objected to claim 8 under 35 U.S.C. §103(a) as being unpatentable over Moloney (WO 96/21029) in view of McCaman et al. (J. Biol. Chem. 261:15345-15348). We respectfully disagree with the Examiner for the reasons that follow.

As stated above, Moloney does not describe a method as claimed in the present application. Claim 8 depends from claim 7 which depends from claim 1 and therefore it carries with it all of the novel features of claim 1. The deficiencies in Moloney are not remedied by McCaman that teaches that the zymogen form of chymosin is activated at pH2 to form a pseudo chymosin product and is further processed to chymosin at a pH of 4.5. McCaman in no way teaches or remotely suggests a method of preparing a recombinant polypeptide as in the claims of the present application.

In view of the foregoing, we respectfully request that the objection to the claims under 35 U.S.C. §103 be withdrawn.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

In view of the foregoing, we submit that the application is in order for allowance and an early indication to that effect would be greatly appreciated. Should the Examiner like to discuss the matter, he is kindly requested to contact Micheline Gravelle at 416-957-1682 at his convenience.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Abstract:

Please insert the Abstract enclosed herewith into the application.

In the Disclosure:

On page 1, after the title of the invention, the following paragraph has been inserted:

--This application claims the benefit under 35 USC §119(e) from U.S. provisional application No. 60/044,254 filed April 25, 1997, now abandoned, which is incorporated herein by reference.--

In the Claims:

Claims 1, 5 and 27 have been amended as follows.

1. (Amended) A method for the preparation of a recombinant polypeptide comprising

a) [introducing into] transforming a host cell an expression vector comprising:

(1) a nucleic acid sequence capable of regulating transcription in a host cell, operatively linked to

(2) a chimeric nucleic acid sequence encoding a fusion protein, the chimeric nucleic acid sequence comprising (a) a nucleic acid sequence encoding a pro-peptide derived from an autocatalytically maturing zymogen, linked in reading frame to (b) a nucleic acid sequence heterologous to the pro-peptide and encoding the recombinant polypeptide, wherein the heterologous nucleic acid sequence is located immediately downstream of the nucleic acid sequence encoding the pro-peptide; operatively linked to

(3) a nucleic acid sequence encoding a termination region functional in said host cell,

b) growing the host cell to produce said fusion protein; and

c) altering the environment of the fusion protein so that the pro-peptide is cleaved from the fusion protein to release the recombinant polypeptide.

5. (Amended) A method according to claim 1 wherein the recombinant polypeptide is hirudin or carp growth hormone.

27. (Amended) A chimeric nucleic acid sequence according to claim 26 wherein the chimeric sequence is as shown in SEQ.ID.NO 1. or SEQ. ID. NO.[2] 3.